

WHAT IS CLAIMED IS:

1 1. A method of identifying a gene or genes associated with a selected
2 phenotype, the method comprising the steps of:

3 (a) providing a nucleic acid library comprising nucleotide sequences that
4 encode at least partially randomized zinc finger proteins;

5 (b) transducing cells with expression vectors, each comprising a nucleotide
6 sequence from the library;

7 (c) culturing the cells so that zinc finger proteins are expressed in the cells,
8 wherein the zinc finger proteins modulate gene expression in at least some of the cells;

9 (d) assaying the cells for a selected phenotype and determining whether or
10 not the cells exhibit the selected phenotype; and

11 (e) identifying, in cells that exhibit the selected phenotype, the gene or
12 genes whose expression is modulated by expression of a zinc finger protein, wherein the
13 gene so identified is associated with the selected phenotype.

1 2. The method of claim 1, wherein the zinc finger protein has three,
2 four, or five fingers.

1 3. The method of claim 1, wherein the library comprises no more than
2 10^7 clones.

1 4. The method of claim 1, wherein the cells are physically separated,
2 individual pools of cells and each individual pool of cells is transduced with an
3 expression vector comprising a nucleotide sequence from the library.

1 5. The method of claim 4, wherein the physical separation of the
2 pools of cells is accomplished by placing each pool of cells in a separate well of a 96,
3 384, or 1536 well plate.

1 6. The method of claim 4, wherein the cells are assayed for the
2 selected phenotype using liquid handling robots.

1 7. The method of claim 1, wherein the cells are pooled together and
2 transduced in a batch.

- 1 8. The method of claim 7, wherein the cells are assayed for the
2 selected phenotype using flow cytometry.
- 1 9. The method of claim 1, wherein the library is made by finger
2 grafting, DNA shuffling, or codon doping.
- 1 10. The method of claim 1, wherein the zinc finger proteins are fusion
2 proteins comprising a regulatory domain.
- 1 11. The method of claim 10, wherein the zinc finger proteins are fusion
2 proteins comprising at least two regulatory domains.
- 1 12. The method of claim 10, wherein the regulatory domain is selected
2 from the group consisting of a transcriptional repressor, a methyl transferase, a
3 transcriptional activator, a histone acetyltransferase, and a histone deacetylase.
- 1 13. The method of claim 10, wherein the regulatory domain is VP16 or
2 KRAB.
- 1 14. The method of claim 1, wherein modulation of gene expression is
2 repression of gene expression.
- 1 15. The method of claim 1, wherein modulation of gene expression is
2 activation of gene expression.
- 1 16. The method of claim 1, wherein the cells are selected from the
2 group consisting of animal cells, plant cells, bacterial cells, protozoal cells, or fungal
3 cells.
- 1 17. The method of claim 1, wherein the cells are mammalian cells.
- 1 18. The method of claim 1, wherein the cells are human cells.
- 1 19. The method of claim 1, wherein expression of the zinc finger
2 proteins is controlled by administration of a small molecule.
- 1 20. The method of claim 19, wherein the small molecule is
2 tetracycline.

1 21. The method of claim 1, wherein the expression vectors are a viral
2 vector.

1 22. The method of claim 21, wherein the expression vectors are a
2 retroviral expression vector, a lentiviral expression vector, an adenoviral expression
3 vector, or an AAV expression vector.

1 ~~23. The method of claim 1, wherein the selected phenotype is related to~~
2 ~~cancer, nephritis, prostate hypertrophy, hematopoiesis, osteoporosis, obesity,~~
3 ~~cardiovascular disease, or diabetes.~~

1 24. The method of claim 1, wherein the zinc finger proteins comprise a
2 Zif268 backbone.

1 25. The method of claim 1, wherein genes that are associated with the
2 selected phenotype are identified by comparing differential gene expression patterns in
3 the presence and absence of expression of the zinc finger protein.

1 26. The method of claim 25, wherein differential gene expression
2 patterns are compared using an oligonucleotide array.

1 27. The method of claim 1, wherein genes that are associated with the
2 selected phenotype are identified by using zinc finger proteins from the library of
3 randomized zinc finger proteins to probe YAC or BAC clones.

1 28. The method of claim 1, wherein genes that are associated with the
2 selected phenotype are identified by scanning genomic sequences for target sequences
3 recognized by zinc finger proteins from the library of randomized zinc finger proteins.

1 29. The method of claim 1, wherein genes that are associated with the
2 selected phenotype are identified by cross-linking the zinc finger protein to DNA with
3 which it is associated, followed by immunoprecipitation of the zinc finger protein and
4 sequencing of the DNA.

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